Long-Term and Ontogenetic Patterns of Heavy Metal Contamination in Lake Baikal Seals (Pusa sibirica)

Ted Ozersky
Mikhail V. Pastukhov
Amanda E. Poste
Xiu Y. Deng
Marianne V. Moore
mmoore@wellesley.edu

Follow this and additional works at: http://repository.wellesley.edu/scholarship

Version: Publisher's version

Recommended Citation
Ted Ozersky, Mikhail V. Pastukhov, Amanda E. Poste, Xiu Y. Deng, and Marianne V. Moore. "Long-Term and Ontogenetic Patterns of Heavy Metal Contamination in Lake Baikal Seals (Pusa sibirica)”. Environmental Science & Technology DOI: 10.1021/acs.est.7b00995
Long-Term and Ontogenetic Patterns of Heavy Metal Contamination in Lake Baikal Seals (*Pusa sibirica*)

Ted Ozersky,*†∥ Mikhaïl V. Pastukhov,‡ Amanda E. Poste,§ Xiu Y. Deng,‖ and Marianne V. Moore‖

†Large Lakes Observatory, University of Minnesota Duluth, Duluth, Minnesota 55812, United States
‡Vinogradov Institute of Geochemistry, Siberian Branch of the Russian Academy of Sciences, Irkutsk, Russia
§Norwegian Institute for Water Research, Oslo, Norway
‖Department of Biological Sciences, Wellesley College, Wellesley, Massachusetts 02481, United States

Supporting Information

**ABSTRACT:** Little is known about the history of heavy metal pollution of Russia’s Lake Baikal, one of the world’s largest lakes and a home to numerous endemic species, including the Baikal Seal, *Pusa sibirica*. We investigated the history of heavy metal (V, Cu, Zn, Cd, Hg, Tl, Pb, U) pollution in Lake Baikal seals over the past 8 decades. C and N stable isotope analysis (SIA) and laser-ablation ICP-MS of seal teeth were used to examine changes in feeding ecology, heavy metal levels associated with life history events and long-term variation in metal exposure. SIA did not suggest large changes in the feeding ecology of Baikal seals over the past 80 years. LA-ICP-MS analyses revealed element-specific ontogenetic variability in metal concentrations, likely related to maternal transfer, changes in food sources and starvation. Hg and Cd levels in seals varied significantly across the time series, with concentrations peaking in the 1960s - 1970s but then declining to contemporary levels similar to those observed in the 1930s and 1940s. Trends in atmospheric emissions of Hg suggest that local sources as well as emissions from eastern Russia and Europe may be important contributors of Hg to Lake Baikal and that, despite the size of Lake Baikal, its food web appears to respond rapidly to changing inputs of contaminants.

**INTRODUCTION**

Fossil fuel combustion, mining, metallurgy, and manufacturing have significantly increased the amount of toxic heavy metals circulating in the biosphere since the industrial revolution.¹⁻³ In many places, environmental concentrations of some metals are high enough to represent risks to wildlife and human health.³⁻⁵ Although areas close to sources of heavy metal emissions tend to be more contaminated, long-range atmospheric transport can result in elevated levels of some metals even in remote regions.⁶⁻⁹ Metals that potentially biomagnify (e.g., Hg, Cd) are of special concern, since they can reach high concentrations in upper trophic levels and impact animals that are ecologically important or are consumed by humans.

Managing risks associated with metal pollution requires understanding the sources and behavior of these elements, as well as how their concentrations vary through time in relation to environmental change. Sediment and ice cores⁶⁻⁹⁻¹³ provide valuable information about inputs and sources of heavy metals into specific areas. Other long-term recorders of heavy metal contamination are hard animal tissues such as bivalve shells, hair, feathers, or teeth, which have been used to describe trends in heavy metal concentrations in clams, birds, polar bears, beluga whales, pinnipeds, and humans.¹⁴⁻¹⁸ Analysis of animal tissues can illuminate a different facet of long-term trends than that revealed by “passive” recorders such as ice cores, because concentrations in animal tissues reflect not only concentrations in the environment, but also patterns in food web transfer and animal life history.¹⁹,²⁰ Importantly, and unlike data from “passive records”, potential toxicological impacts can be deduced from concentrations in archival tissue when such concentrations are linked directly to those in soft tissue and to toxicological thresholds.²¹ Information about inputs to ecosystems may also be gleaned from animal tissue records if life history and food web factors (such as age and trophic position) are considered.¹⁷,²⁰,²¹

In Russia’s Lake Baikal, the top consumer is the Baikal seal (*Pusa sibirica*), the world’s only true freshwater seal. Baikal seals are culturally and economically important in the Baikal region, and have long been hunted for furs and food by humans living on the shores of the lake.²² The earliest measurements of heavy
metal concentrations in Baikal seals (or any other compartment of the Lake Baikal food web) were carried out in the early 1990s, and appear to have remained relatively stable since then at levels that do not pose risks to the seals or their human consumers. It is unknown, however, how recent metal concentrations in Baikal seals and the Lake Baikal food web in general compare with historic values throughout the 20th century, a period marked by global and regional environmental change. Aside from helping to reconstruct the pollution history in Lake Baikal, examination of past trends in metal contamination may help determine whether the food web of this large and remote lake is sensitive to potential future changes in heavy metal inputs.

Various anthropogenic sources could have contributed heavy metals to Lake Baikal throughout the 20th century. Regional sources include gold mining in the watershed (beginning in the 19th century), combustion of fossil fuels and emissions from industry. Atmospheric transport from industrial centers in the Eastern USSR, Europe and further afield is another potential pathway for transport of mobile elements like Hg, Cd, Pb, and Tl. Understanding historical patterns and sources of metal contamination to Lake Baikal is also important in the context of recent accelerated environmental change in the region. Increasing frequency of forest fires in Siberia, continuing increases in Hg emissions in China and India, intensifying mining along tributaries of Lake Baikal in Mongolia, eutrophication in Lake Baikal and climate change can all potentially change inputs of metals to the lake or their transfer through the food web.

Our primary goal was to determine the recent history of metal pollution in Lake Baikal’s top predator. We used an archival collection of Baikal seal teeth together with δ13C and δ15N stable isotope analysis (SIA) and laser ablation ICP-MS to answer three questions: (1) Did the trophic level or food sources of the Baikal seals change over the last 80 years? (2) Are there ontogenetic and seasonal patterns in the concentrations of V, Zn, Cu, Cd, Hg, Pb, Tl, and U in Baikal seal teeth? (3) Did levels of these metals change over the last 80 years in Baikal seals? We address these questions in the context of documented environmental change in the Lake Baikal region, the life history of the Baikal seal and emission trends in potential source areas of pollution.

### MATERIALS AND METHODS

#### Study Site and Background

Located in central Siberia, Lake Baikal—the deepest and oldest lake in the world—is host to hundreds of endemic species, including the Lake Baikal seal (Supporting Information Figure S1). Baikal seals are born in spring in ice caves, feed on milk for the first 2–2.5 months of their life, and then transition to an adult diet, composed primarily of the endemic sculpins Comephorus and Copetcomephorus spp. Baikal seals actively feed throughout most of the year, but food intake is lower in winter and almost stops during a 4–6 week period of starvation in late spring associated with mouling and sun basking, during which seals can lose almost all their fat reserves. Baikal seals are long-lived, with all their fat reserves. Baikal seals are long-lived, with all their fat reserves.

#### Sample Collection and Processing

Seals were collected between 1965 and 2013. Between 1965 and 1989, seals were collected mostly in central Baikal by Russian researchers working with commercial seal hunters, retaining skulls and teeth for morphometric analyses. Sampling was more intensive in some years than others; of the seals used in this study 19, 28, and 14 were collected in the 1960s, 1970s and 1980s, respectively. In 2013, 22 seals were collected and dissected in the field in southern and central L. Baikal under scientific-collection permit No. 032013031058 issued to M. Pastukhov by the Russian Federal Fisheries Agency. Upper canines were removed from seal skulls and cleaned to remove external debris. Teeth were mounted on wooden blocks with epoxy resin (Epofix, Struers) and sliced longitudinally on a low speed IsoMet saw (Buehler) with a diamond wafering blade to form 2 mm thick slices centered on the tooth midline (Figure 1). One slice was used for δ13C and δ15N SIA, the other for LA-ICP-MS analysis.
δ13C and δ15N SIA. Dentine for δ13C and δ15N SIA was removed from tooth slices using a Dremel tool (Robert Bosch GmbH) with a fine, carbide-tipped dental bit. Approximately 20 mg of dentine were drilled separately from the prenatal and first translucent dentine (corresponding to the first period of independent feeding) layers of teeth from 56 seals. We recovered dentine from only these two layers because subsequent layers were typically too narrow to sample with our approach. Dentine was decarbonated using 10% HCl, centrifuged and washed, leaving behind mostly collagen.35,36 In addition to dentine collagen, soft tissue samples collected from the pulp cavities (Figure 1A) of teeth from 51 seals were analyzed for SI. Pulp cavity material was comprised mainly of dried blood and blood vessels, and its SI values should represent those over the last few months of the life of the animal before capture. %C and %N values were used to evaluate whether tissue spoilage and changes to δ13C and δ15N SI values had occurred during sample storage.34 Samples with unusual %C and %N (>3 SD from mean) were excluded from analysis; of the 192 samples analyzed 37 were excluded on this basis. SIA was performed at the UC Davis Environmental Stable Isotope Lab. Every fifth sample was analyzed in duplicate; differences between duplicate samples ranged up to 0.56‰ (average = 0.2% ‰) for δ13C and 0.67‰ (average = 0.37% ‰) for δ15N. Trends in SI values from seal dentine were compared with published δ13C and δ15N SI values37 for omul scales (Baikal whitefish, Coregonus automnalis migratorius), spanning the period between 1947 and 1995. Omul scale SI data were extracted from Figures 3 and 4 of Ogawa et al.37 using WebPlotDigitizer.38 All seal and omul δ13C values were Suess-corrected19 to account for increased 13C in the atmosphere due to fossil fuel combustion; 1966 was used as the "baseline" for corrections.

LA-ICP-MS Analysis. Tooth sections for LA-ICP-MS analysis were cleaned by rinsing in ultrapure deionized water and trace metal clean 5% nitric acid. Analysis was conducted at the Woods Hole Oceanographic Institution Plasma Mass Spectrometry Facility on a high-resolution magnetic sector Element2 ICP-MS (Thermo Fisher Scientific) coupled to a 193 nm excimer laser ablation system (Electro Scientific Industries). LA-ICP-MS run parameters are shown in Table S1. Between 2 and 10 spots per tooth (depending on animal age and tooth thickness; Figure 1) were analyzed, starting from prenatal dentine and moving toward the pulp cavity. In total, teeth of 45 seals were analyzed. Analysis targeted 51V, 63Cu, 64Zn, 114Cd, 203Hg, 205Tl, 208Pb, and 238U. These metals were selected because of their toxicity and association with anthropogenic pollution. 48Ca was used as an internal standard,4,44 with metal concentrations expressed as a ratio of counts-per-second relative to 48Ca. Concentrations of 48Ca (as CPS) were consistent in different layers of teeth, varying minimally within and across teeth compared to concentrations of target metals. NIST612 glass was used as an external standard. Instrument blanks (carrier gas and 2% HNO3) and the NIST612 standard were analyzed at the start and end of a run for each tooth. On average, element/48Ca ratios of duplicate NIST612 analyses (at start and end of each sample run) differed by 2.2%, 4.3%, 6.9%, 9.6%, 23.1%, 20.4%, 20.4%, and 21.9% for 51V, 63Cu, 64Zn, 114Cd, 203Hg, 205Tl, 208Pb, and 238U, respectively. Drift in blanks and standards was accounted for by assuming linear change during analysis of each tooth.44 Final results were expressed as the ratio of counts-per-second (CPS) for each element to 48Ca, standardized (relative) to NIST612 CPS. While this approach does not allow for determination of absolute concentrations of metals in teeth, it ensures that results are consistently expressed relative to a homogeneous standard, allowing comparison of metal concentrations across samples. Results for spots with high variability in metal concentrations (>10% relative standard deviation in CPS) during ablation were excluded from analysis on the assumption that the laser encountered another dentine layer or impurities during ablation; less than 3% of observations were excluded in this way. Ablated tooth sections were examined under an optical transmission microscope with polarized light and ablated spots were assigned to dentine layers. Data from spots that obviously overlapped layers (e.g., fourth spot from top in Figure 1B) were not included in the final analysis; multiple samples from the same layer (e.g., fifth and sixth spot from top in Figure 1B) were aggregated by averaging.

Data Analysis. δ13C and δ15N Values. GAMMs (generalized additive mixed models) were used to model trends in δ13C and δ15N stable isotope ratios in seal tooth pulp and dentine using the mgcv package41 in the R statistical computing environment.42 GAMMs allow fitting of nonlinear splines while incorporating random effects and nesting.42 Models were parametrized to fit smoothers to δ13C and δ15N values for all seal tissues (prenatal dentine, translucent dentine, pulp tissue) with year of sample (year corresponding to the formation of a dentine layer or year of animal death for pulp tissue), tissue type, gender and age-at-death as fixed predictor variables and seal ID as a random variable. Models were simplified by sequentially dropping nonsignificant predictor variables, until all remaining variables contributed significantly to the model (α = 0.05). Diagnostic plots were used to assess the assumptions of normal distribution and homogeneity of variance of residuals; assumptions were met in all cases and no transformations were needed.

Metal Concentrations in Dentine. Repeated-measures, linear mixed-effects ANOVAs, followed by Tukey posthoc tests were used to examine differences in metal concentrations in different dentine layers of teeth to assess ontogenetic and seasonal differences in incorporation of metals into dentine. The repeated-measures, mixed-effects approach was used because different dentine layers are nested within each seal and represent repeated measures for each animal. Analysis was implemented using the nlme44 and multcomp45 packages in R. To account for potential differences in metal concentrations between seals due to temporal trends and to improve normality and variance of residuals, metal concentrations were standardized for each seal to mean = 0 and standard deviation = 1; thus analyses compare relative differences in metal concentrations in different dentine layers. Although up to seven dentine layers were analyzed for some seals, we restricted statistical comparisons to the first five layers, since there were few samples of layers 6 and 7.

GAMMs were used to examine temporal trends in concentrations of metals across the 80 years covered by our data set. Concentration trends of each metal were modeled separately with year of sample, gender and age (<1 year old or >1 year old) as fixed independent variables and dentine layer identity (prenatal, first opaque layer, first translucent layer, etc.) and seal ID as random variables, with layers nested within seal ID. Models were simplified as described for SI GAMM models. Absence of temporal autocorrelations was confirmed by plotting autocorrelation function (ACF) values. Metal data
were normalized to mean = 0 and standard deviation = 1 across all seals to improve normality and variance of residuals; assumptions were assessed using diagnostic plots, and were met in all cases.

Hg Source Attribution. To attempt source attribution of Hg, the most toxic metal to display a significant time trend in the dentine of the Baikal seals, we compiled information about atmospheric Hg emissions in potential source regions. We compiled data on estimates of atmospheric Hg emissions from four different regions in the Northern Hemisphere and coal combustion in the Irkutsk region. Data were extracted for 5-year intervals and normalized on a 0- to 1 scale to eliminate differences in units of measurement. These normalized data were plotted with the spline of normalized data for dentine Hg.

## RESULTS AND DISCUSSION

Seal Trophic Position and Energy Sources. GAMM models on combined seal tissues (pulp material and two types of dentine) showed significant differences in δ13C between tissue types (pulp tissue was more depleted in 13C than both types of dentine) and a significant overall trend in Suess-corrected δ13C values through time, with increase of approximately 1‰ between the 1960s and the present (Figure 2A, B). Increasing δ13C values through time could be due to increased rate of planktonic primary production or increased importance of benthic energy sources to the seals. The first explanation is consistent with inferred increases in primary production in Lake Baikal over past decades, and the magnitude of the increase in δ13C agrees with published chlorophyll – δ13C relationships. Increased importance of benthic carbon in seal diets would also be consistent with enrichment in δ13C, but is less likely. Baikal seals consume mostly golomyanka fish, a strictly pelagic feeder and it is unlikely that diets of golomyanka changed much over time. In any case, the very modest change in seal δ13C values does not indicate major changes to diets.

No significant differences were noted among seal tissue types in δ15N values, but δ15N values showed significant, although not directionally consistent, variation through time (Figure 2C, D). A large “hump” in δ15N values in the late 1980s was mostly driven by samples of pulp tissue from three young seals. Removing these pulp samples did not significantly change the overall shape or significance of the trends. Seal δ15N values were consistently higher than omul scale values, suggesting seals are approximately 1 trophic level above omul, although omul are not an important part of seal diets. Like in seals, omul δ15N values fluctuated through time, but with no directional trend. The observed fluctuations in δ15N values could be due to changes in the feeding patterns and trophic level of seals and omul or variation in the sources of N to primary producers in the system, but separating these two processes is impossible with available data. In any case, the modest increase in δ13C values and the lack of a large variation or consistent directional change in δ15N values do not suggest that the diets of Baikal seals changed much over the last 80 years. This result makes it more likely that temporal trends in dentine metal concentrations (see below) reflect changes in metal inputs into the lake rather than changing food web structure.

Ontogenetic Shifts and Seasonal Patterns of Dentine Metal Concentrations. There were significant variations in metal concentrations in dentine formed in different periods of the lives of Baikal seals, and different metals showed different patterns of variation among dentine layers (Figure 3). Several metals were significantly and strongly correlated with each other when examined across all samples (Spearman’s rank correlation test, Figure S2): Hg concentrations were negatively correlated with V, whereas V, Pb, and U were positively correlated with each other and negatively correlated with Zn. Interpretation of these patterns is not straightforward because the behavior and tissue-partitioning of metals in organisms is complex and highly element-specific, and because studies on the biological behavior of some metals (e.g., Tl) are rare. Additionally, few studies have examined microspatial variation of metals in human and animal teeth and the causes of these variations are not well-understood at present.

Despite uncertainties about the relationship between soft tissue and dentine metal concentrations, some of the microspatial patterns in metal concentrations that we observed agree with what is known about maternal transfer and ontogenetic changes in metal concentrations in mammals. For example, it is well-known that Hg levels in neonatal and very young pinnipeds can be high because the placenta does not form an effective barrier to the transfer of Hg. This agrees with our results, which showed high Hg levels in prenatal dentine and a decrease with age, and with results from analysis of soft tissues of contemporary Baikal seals. The behavior of Tl has not been studied in pinnipeds, but the low levels in prenatal dentine and subsequent increase in our samples may be due to the low permeability of the placenta to Tl (as has been shown for rats), followed by Tl accumulation from food.
or water after birth. The maternal transfer of V, U, and Pb has been well established, but the placenta acts as a partial barrier to these metals and their levels are lower in newborn mammals than in their mothers, which may account for relatively low levels of these metals in prenatal and opaque dentine formed early in life in Baikal Seals. Wagemann et al. observed higher levels of Zn and Cu in tissues of seal pups than in tissues of their mothers, attributing it to higher nutritional requirements for these elements by young seals, which may account for the higher levels of Cu and Zn we observed in dentine formed early in life.

Placental Cd transfer is not well understood, but appears to be lower than for Hg, which may account for the lack of difference in Cd concentrations between prenatal dentine and dentine laid down later in life. The slow (but not significant) increase in Cd levels in dentine laid down later in life may be due to increased accumulation of this metal from food.

Postnatal seasonal differences in metal concentrations between opaque and translucent dentine may be related to patterns of feeding and starvation by the seals, but less is known about seasonal than early life ontogenetic changes in metal concentrations in marine mammals. Higher concentrations of V, Pb, and U in translucent dentine may indicate higher intake and blood levels of these elements during periods of active feeding, but the lack of a similar pattern for other metals argues against this interpretation. Increased concentrations of Zn in opaque dentine formed during starvation periods are difficult to explain, but may reflect loss of Zn from catabolized muscles to catabolized muscles.

Figure 3. Variation in standardized metal concentrations in different dentine layers of Baikal seal teeth. X-axis labels: PD = prenatal dentine, OD = opaque dentine, and TD = translucent dentine with numerals denoting year of life in which the dentine layer was formed. Gray “spaghetti” lines represent changes for individual seals. Results of mixed model ANOVAs and Tukey posthoc tests performed on values for the first five layers are shown above each figure. Dentine layers sharing a common letter were not significantly different at the 0.05 level (Tukey post hoc test).
the blood and subsequent deposition in dentine. Research on starved turkey poults and rats\textsuperscript{64,65} has shown that Zn levels in many tissues increased during starvation as Zn from skeletal muscle was remobilized. Copper remobilization in young turkeys during starvation followed similar patterns to Zn, but was less pronounced,\textsuperscript{65} which may explain the lack of a strong seasonal pattern in dentine Cu levels.

This is the first study to explicitly characterize variation in metal concentrations in the teeth of an aquatic mammal on a subannual time scale, and our findings of metal-specific variation among different dentine layers call for better understanding of the seasonality of metal concentrations in aquatic mammals and of the relationships between soft tissue and dentine metal concentrations. Improved understanding of the relationships between metal concentrations in the body and dentine\textsuperscript{21} would open new possibilities for exploring seasonal and ontogenetic patterns in metal contamination based on tooth samples in mammals.

Figure 4. Variation in concentrations of eight different metals in Baikal seal teeth across the 80-year time series. Results of GAMM analyses are shown where a significant effect of an independent variable (i.e., year, age, or gender) was detected. Trendlines with a 95% prediction interval are shown only where time trends were significant. Filled circles = adult seals, empty circles = juvenile seals. In E: filled circles = female seals, empty squares = male seals.
Long-Term Trends in Metal Concentrations. We found significant temporal changes for standardized concentrations of Hg and Cd in Baikal seal dentine over an 8-decade period (Figure 4A, B). Concentrations of Hg and Cd were low in the 1930s and 1940s, increased in the 1950s to peak concentrations through the 1960s and early 1970s and then decreased and stabilized at levels comparable to those of the 1930s and 1940s. It is interesting to note that Hg and Cd are the metals most likely to biomagnify among those examined.5,6,10 Although no significant trends were observed for U or Pb, their highest concentrations were also observed in the 1960s and 1970s (Figure 4C, D). There were no significant time trends for other metals. However, Tl levels were higher in male than female seals (Figure 4E), with a weak and borderline-significant downward trend through time in males, a pattern that we struggle to explain. Levels of V, Zn, and Cu were significantly higher in juvenile than adult seals. As stated earlier, it is well established that Zn and Cu levels are higher in juveniles than adult mammals, but we are unsure how to explain the pattern for V.

The trends we observed for Hg and Cd are in general agreement with a wide range of studies tracking temporal changes in atmospheric inputs of metals to lake sediments and glacier ice, concentrations in archival animal tissues and inventories of atmospheric emission of metals.9−12,14,17,47,68−71 Studies reconstructing metal inputs into glaciers and sediments have documented large increases in atmospheric transport and deposition of many metals associated with coal combustion and metallurgy (Hg, Cd, Zn, Cu, and Tl among others) and of Pb, associated mainly with leaded gasoline use, throughout the first half of the 20th century, including in the Lake Baikal region.10,47 As we have shown for Lake Baikal, some regions experienced declines in atmospheric metal inputs starting in the last decades of the 20th century, presumably due to improved pollution controls, reduced use of leaded gasoline and economic forces.8,11−14 Studies utilizing archival animal tissues to reconstruct metal inputs into aquatic environments have also often shown increasing concentrations of heavy metals through the 20th century, followed by declines in some locations. For example, Pb levels in shells of Atlantic clams near the coast of the U.S. closely tracked the rise and eventual decline of U.S. lead emissions from gasoline.14 Levels of Hg in archival collections of bird feathers, polar bear and arctic seal teeth also track changing atmospheric Hg emissions in potential source regions.13,17,20

An interesting question is why we observed temporal changes in levels of some metals associated with fossil fuel combustion (Hg, Cd), but not others (V, Cu, Zn, Tl, Pb, U). One possible explanation is the different susceptibility of metals to volatilization during coal combustion and hence long-range atmospheric transport. For example, Hg is especially prone to volatilization and hence very long-range transport,72 which may mean Baikal receives Hg from more sources than other elements. The idiosyncratic behavior of different metals in organisms (e.g., Cu and Zn are biologically essential elements and may be under stricter physiological than environmental control) may offer another explanation for the lack of concordance in concentration trends of metals associated with fossil fuel emissions.

Emission trends of Hg and other metals often follow distinct regional trajectories (Figure 5). For example, both the peak and the decrease in atmospheric Hg emissions in Europe and the
Hg to Lake Baikal\textsuperscript{56} may mean that Hg inputs will stabilize as well. However, increased mining and industrial development in the watershed and airshed or changes to the hydrological regime and food web structure may lead to changing levels of heavy metals in the Baikal food web. Long-term monitoring of inputs and concentrations of metals and other contaminants in different compartments of the Baikal ecosystem is needed to ensure protection of this unique lake.

\section*{REFERENCES}

\section*{ACKNOWLEDGMENTS}

The pioneering research and scientific collections of Baikal seals conducted by Vladimir Pastukhov made this study possible and warrant special recognition. We thank Kate Corcoran, Abby Duck, Jenna Disch, Graham Durovich, Amanda Gardner, Gabi Guzman, Julia Halbur, Diana Lee, Iris Lin, Kirill Shchapov, and Melaina Wright for help with sample processing, data and LA-ICP-MS analyses. Comments by three anonymous reviewers helped to substantially improve this manuscript. This work was supported by a Brachman-Hoffman Fellowship, a Brachman-Hoffman Small Grant, and a Fiske grant from Wellesley College in addition to the Dimensions of Biodiversity Program of the US National Science Foundation (DEB-1136657).

\section*{AUTHOR INFORMATION}

Corresponding Author

*E-mail: tozersky@d.umn.edu.

ORCID

Ted Ozersky: 0000-0002-1842-7745

Notes

The authors declare no competing financial interest.
(42) RDC Team R: A language and environment for statistical computing 2015.


(64) Bremner, I.; Davies, N. T. The induction of metallothionein in rat liver by zinc injection and restriction of food intake. *Biochem. J.* 1975, 149 (3), 733–738.